ORIGINAL ARTICLE

Saliva contamination effects at different application steps on bond strength of total etch two-step adhesive

Efeitos da contaminação por saliva em diferentes etapas de aplicação do sistema adesivo convencional de dois passos na resistência de união de restaurações adesivas

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Abstract

Introduction: there are clinical situations in the management of cavities favorable to saliva contamination. Human saliva is a complex mixture of oral fluids and it has been showed that the protein content of the saliva is responsible for the decrease in adhesive bond strength when contamination has occurred. **Objective**: this study aimed to evaluate the effect of saliva contamination during different steps of application of adhesive system on bond strength of total etch two-step adhesive system. **Methods**: twenty-five extracted human molars were ground flat to expose occlusal dentin. The bonding system and composite resins were applied to the dentin after etching step under five conditions (n=5/group): G1: control group–no contamination; G2: etching/contamination/dry/adhesive system application; G3: etching/contamination/wash/dry/adhesive system application; G4: etching/adhesive system application/contamination/wash/dry/adhesive system re-application; and G5: etching/adhesive system application/contamination/dry. Microtensile bond strength was tested after specimens were stored in distilled water at 37ºC for 24h. Data (mean values -μTBS) were analyzed by ANOVA one-way and Tukey tests (α=5%), respectively. Results: Groups G2, G3 and G4 revealed bond strength similar to the control (G1). Group G5 showed significantly lower bond strengths than other groups (p<0.001). **Conclusion**: the total etch two-step adhesive tolerated salivary contamination except when the contamination occurred after application of the bond and it was only removed with an air jet and adhesive system was not reapplied.

Key words: Saliva. Dentin.

Resumo

Introdução: a saliva humana é uma mistura complexa de fluidos orais e tem sido demonstrado que o conteúdo proteico da saliva é responsável pela diminuição da resistência adesiva quando ocorre contaminação. **Objetivo:** este estudo objetivou avaliar o efeito da contaminação salivar durante diferentes etapas de aplicação do sistema adesivo sobre a resistência de união. **Métodos:** vinte e cinco molares humanos extraídos foram cortados para exposição da dentina oclusal. O sistema adesivo convencional e as resinas compostas foram aplicados na dentina após a etapa de condicionamento sob cinco condições (n = 5 / grupo): G1: grupo controle - sem contaminação; G2: condicionamento ácido/contaminação/secagem/adesivo; G3: condicionamento ácido/contaminação/lavagem/secagem/adesivo; G4: condicionamento ácido/aplicação do sistema adesivo/contaminação/lavagem/secagem/reaplicação do sistema adesivo; e G5: condicionamento ácido/aplicação do sistema adesivo/contaminação/secagem. A resistência à microtração foi testada após o armazenamento das amostras em água destilada a 37ºC por 24 horas. Os dados (valores médios -μTBS) foram analisados por ANOVA unidirecional e testes de Tukey (α = 5%), respectivamente. Resultados: Os grupos G2, G3 e G4 revelaram resistência de união semelhante ao controle (G1). O grupo G5 apresentou resistência de união significativamente menor que os demais grupos (p <0,001). **Conclusão:** o adesivo convencional de dois passos não foi afetado pela contaminação salivar, exceto quando a contaminação ocorreu após a aplicação adesiva e foi removida apenas com jato de ar e o sistema adesivo não foi reaplicado.

Palavras-chave: Saliva. Dentina

INTRODUCTION

Composites and adhesive systems are by far the most widely-used and versatile dental material available to the dental professional[1]. These materials have a wide variety of applications mainly due to their esthetics, and direct-filling capabilities, but there are concerns about their clinical performances in long-term services[2,3]. Secondary caries around restorations restorative margins, fracture and discoloration are the primary modes of resin-based dental materials failure[4]. Deterioration of composite/adhesive-dentin bond interface integrity as a result from poor initial adhesion appear to be largely related to these failures[5].

The resin/dentin bond strength has been shown as the key for suitable clinical performance and many different factors including materials, patient and dentist-related factors[6]. The placement technique, under the control of the clinician, is also

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Conflict of interest: There are no conflicts of interest in this research.
Received: 15 Set 2018; Revised: 5 Mar 2019; 1 Abr 2019; Accepted: 3 Abr 2019

a critical approach to improve adaptation, retention and sealing of the material within the preparation[4]. Performing satisfactory moisture control with no blood, gingival crevicular fluid or saliva contamination is a frequent problem encountered in restorative dentistry daily practice[7]. Clinical situations in the management of cavities with sub gingival margins, partially erupted crowns, malpositioned teeth, or uncooperative patients where rubber dam isolation is not unlikely to be performed are examples of conditions favorable to saliva contamination[8].

Human saliva is a complex mixture of oral fluids mainly composed by water, enzymes, electrolytes and proteins such as mucoproteins, albumin and gama-globulin[9]. It has been showed that the protein content of the saliva is responsible for the decrease in adhesive bond strength when contamination with saliva has occurred[10,11]. Previous studies related to the bonding efficacy of adhesive system steps contaminated with saliva are controversial. Some studies demonstrated that saliva contamination reduces the bond strength of dental adhesives to dentin[12,13] while others reported conflicting results[7,14]. These differences are related to various influencing factors, such as the composition of adhesive systems and the stage of bonding procedures that saliva contamination occurs[15]. Then, it is not possible to conclude plausible orientations to clinicians.

Currently, a popular strategy in adhesive dentistry involves the total etch, two step adhesive system where a single solution contain primer and adhesive components[16]. These systems have become increasingly popular in daily practice in Brazil due to their simplified and thus more user-friendly application procedure[17]. When using this system, the first step involves the application of a phosphoric acid gel, followed by the bond component (second step). These sequential steps are susceptible to saliva contamination during clinical procedures.

The purpose of this study was to evaluate the effects of saliva contamination at different bond application steps on the microtensile bond strength of a two-step total-etch adhesive systems and to identify strategic additional steps in bonding procedures that reestablishes bond strength comparable with the non-contaminated control group. The null hypothesis tested was that saliva contamination at different stages during the bonding procedures has no significant effect on the dentin bond strength of a two-steps total-etch adhesive system.

MATERIAL AND METHODS

The materials used in this study included one 37wt%- phosphoric acid and one(Condicionador Dental Gel, Dentsply. International, York, USA) commercial two-step total-etch adhesive Adper™ Single Bond 2 (3M/ESPE, St. Paul, USA), and Z-100 (3M/ESPE, St. Paul, USA). The chosen adhesive system contains BisGMA (Bisphenol A-Glycidyl-Methacrylate), HEMA (Hydroxyethyl Methacrylate), dimethacrylates, ethanol, water, photoinitiator system and a methacrylate functional copolymer of polyacrylic and polyitaconic acids with 10% by weight of 5 nm-diameter spherical silica particles as basic composition.

The Ethical Research Committee of the School of Medicine of the Federal University approved the use of 25 extracted caries-free human third molars in this study (protocol # 050/11). The teeth were stored in 0.01% (w/v) thymol solution at 4°C and used within 3 months after extraction[18]. A flat dentin surface was exposed on each tooth after wet grinding of the occlusal enamel on #100-grit SiC paper mounted in a polishing machine (Arotec SA, Cotia, Brazil). Dentin surfaces were exposed and inspected under ×8 magnification to ensure that no enamel remnants were left (Leica Zoom 2000 - Leica Microsystems GmbH, Wetzlar, Germany). The enamel-free exposed dentin surfaces were further polished on wet #600-grit silicon-carbide paper for 20 s to produce a standardized smear layer. Specimens were etched with 37% phosphoric acid gel for 15 s, rinsed with water and dried with absorbent paper.

Using a computer-generated list, we randomly assigned the teeth to one of 5 groups (n=5), according to the saliva contamination during bonding procedures as showed in Figure 1. The sample size calculation was based in a previous pilot study. A description of all groups is as follows:

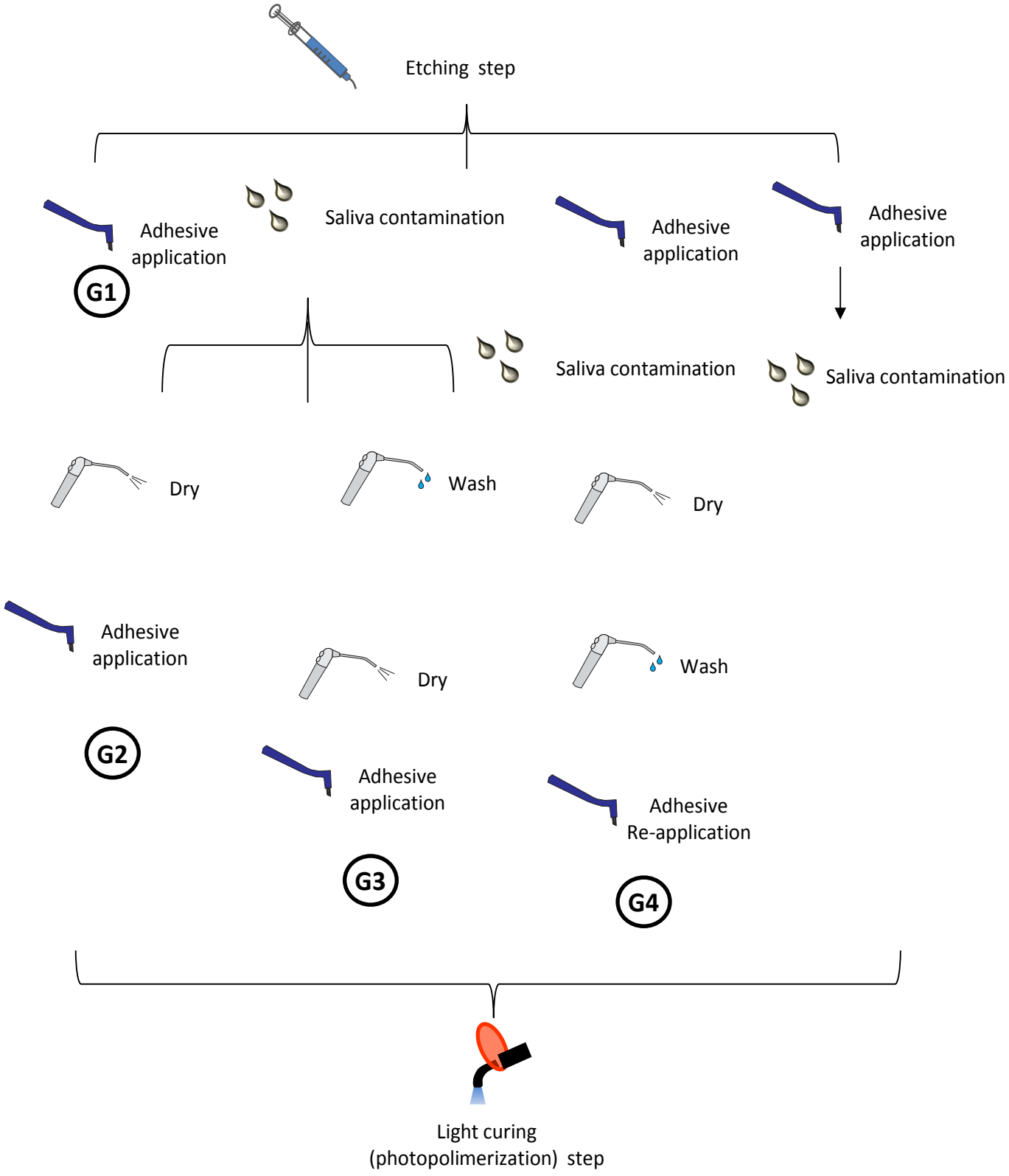
- G1 (Control): In this group, there was no saliva contamination. The adhesive system was applied to the dentin of each specimen according to the manufacturer's instructions (Apply two consecutive coats of adhesive for 15 s with gently agitation, gently air thin for 5 s to evaporate the solvent and light cure for 10 s)
- G2 (Contamination/ dry/ adhesive system application): fresh human saliva was applied with a disposable brush to the etched dentin for 20 s. Surface was then dried with air for 5 s and the bonding agent applied as in the control group.
- G3 (Contamination/ wash/ dry/ adhesive system application): After saliva contamination of etched dentin, the surface was rinsed with water for 5 s, and it was then dried with air for 5 s. Then, the bonding procedure was carried out as in the control group.
- G4 (adhesive system application/ contamination/ wash/ dry/ adhesive system): After the bonding agent application, saliva decontamination was performed, the surface was rinsed with water for 5 s and dried with air for 5 s. The bonding agent application was repeated.
- G5 (adhesive system application/ contamination/ dry): After the bonding agent application, saliva contamination was performed, the surface was dried with air for 5 s. None additional procedures were carried out.

For all groups, the adhesive was light activated for 10 s using a Light Emitting Diode Optilight LD Max (Gnatus, Ribeirão Preto, Brazil) with a power density of 460 mW/cm².

The fresh saliva used in the study was provided by the researcher himself in the act of the restorative process, being collected one hour after meal, thus simulating a clinically existing situation. The time and the amount of saliva contamination were determined by previous study of Darabi et al[19]. All the

experimental procedures were performed by a single operator to reduce inter-operator variation. Dentin surfaces were bonded according each group. Then, composite resin was then used incrementally to build up the specimen to a thickness of 4 mm. Each 2-mm increment was individually light-activated for 40 s. The specimens were stored in deionized water at 37°C for 24 h.

Figure 1. Schematic representation of the experimental design with group representation. The steps at which saliva contamination occurred during the bonding procedure and the strategic steps to overcome the contamination are illustrated.



Microtensile bond strength test (μTBS)

The bonded teeth were serially sectioned, with a water-cooled diamond saw in a cutting machine, in mesial-distal and buccal-lingual directions, to obtain sticks with a cross-sectional area approximately 0.9 mm^2, measured with a digital caliper (Absolute Digimatic, Tokyo, Japan). Each stick was glued with cyanoacrylate-based adhesive (Zapit Base and Accelerator, Dental Ventures of America Inc., Corona, USA), attached to opposing arms of the testing device and finally stressed until failure with a tensile force in a microtensile testing machine (Model 4440, Instron Corp., Canton, USA) at a crosshead speed of 1 mm/min. The bond strength (MPa) of each specimen was determined as the failure load (N) divided by the cross-sectional area of the bonded interface.

Failure pattern determination

Both surfaces of each fractured specimen were observed under a stereomicroscope (Leica Zoom 2000 - Leica Microsystems GmbH, Wetzlar, Germany) at ×80 magnification to record the types of failure, which were classified as mixed, cohesive failure in dentin, and cohesive failure in composite resin.

Statistical Analysis

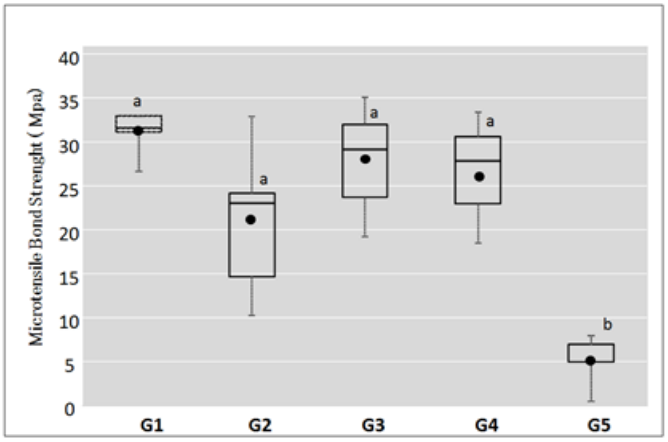
The experimental unit in this study was the tooth and values of all sticks from the same tooth were averaged. For statistical purposes, an arbitrary value corresponding to approximately half of the minimum μTBS that could be observed in this study (5.0 MPa) was attributed to specimens that spontaneously debonded (premature debond failure) during cutting procedures[20]. The normality of error distribution and the degree of non-constant variance were checked for each response variable using Shapiro-Wilk test. Statistical analysis was carried out using the SPSS software package (SPSS Software, version 18.0, SPSS Inc, Chicago, IL, USA). Comparison of the μTBS of the five studied conditions was performed using one-way analysis of variance (ANOVA) and Tukey tests.

RESULTS

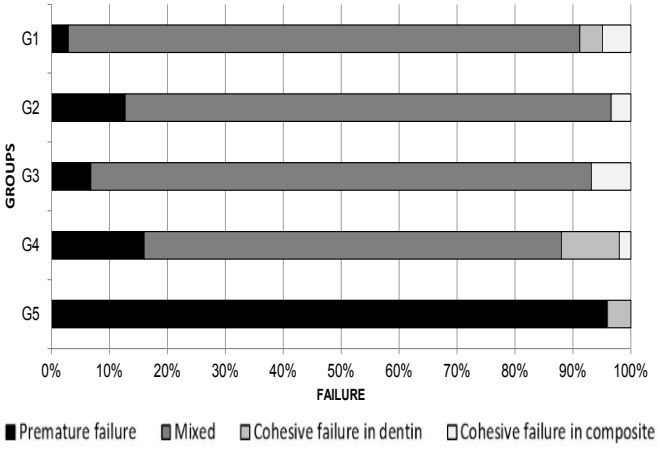
The mean μTBS and respective standard deviations are summarized in Graphic 1. A one-way ANOVA found a significant effect for studied conditions ($p<0.001$). The Tukey test ($p<0.05$) revealed significant differences for group G5 related to saliva contamination after adhesive application with just air dry like overcoming strategy presented the lowest bond strength, which was different from that of all other groups. The highest μTBS was found for the control group, in which there were no significant different comparatively to groups G2, G3 and G4, that had additional strategic steps (drying, rinsing or re-application of adhesive layer) were performed.

Graphic 2 displays the fracture pattern failure as well as the premature failure related to the groups studied. The results of fracture mode analysis showed that mixed fractures were prevalent in G1 (control) group and in those groups wich some strategic step was performed. In contrast, the group subjected to saliva contamination prior the adhesive application (G5) were tremendously affected (94 % of premature failure) by this condition.

Graphic 1. Box-whisker plots of the microtensile bond strength in MPa for the different experimental groups tested. The median μTBS is represented by the central line and the mean by black dot. The box represents the interquartile range. Groups identified with different letters were statistically significantly different ($p<0.05$).



Graphic 2. Percentage of fracture-type and premature debonded specimens in each group.



DISCUSSION

This study evaluated the effect of saliva contamination during different application steps and possible procedures to overcome the contamination on the bond strength of etch-and-rinse two steps adhesive systems to dentin. The null hypothesis of the present study that saliva contamination could not interfere on bond strength was rejected. The group submitted to contamination after adhesive application, in which only air-drying was done, revealed a significantly lower bond strength mean values corresponding to a reduction of 83% in relation to control; however, there were not significant differences in μTBS among the other groups when the contamination occurred but

additional strategic steps (blot-dry, wash off, the association blot-dry and wash off, and re-application of bonding agent) were applied to remove the contaminating agent ($p>0.05$). In these groups the reduction on bond strength range from 11-32% in relation to control. These findings are consistent with literature data[19,21].

In a previous study using the same adhesive system, evaluated through shear bond test, the effect of saliva contamination during bonding steps without procedures to remove saliva showed great impact of contamination after adhesive application and before its polymerization. They found bond strength values for this condition about 50% in relation to control[22]. It has been suggested that the water content from saliva can affect the degree of conversion and bond strength. Molecules from HEMA monomer present great hydrophilicity and may retain water within the adhesive layer with no participation in chain growth during polymerization[23]. This induces an inhomogeneous structure of the cured adhesive, which might be a potential mechanism for degradation[24]. In addition, the glycoproteins present in saliva lead to an adherent organic coating that may prevent complete infiltration of the next composite layer and lead to an insufficient copolymerization[25]. This kind of contamination seems to be a potential cause for poor bond quality of adhesive systems during restorative procedures[26].

Interestingly, the use of simple strategic procedures on the saliva-contaminated surfaces could satisfactorily recover the bond strength. Water-rinsing associated to air-drying procedures (G3) greatly restored the μTBS, which is consistent with previous study[27]. Air-drying strategic step was less effective to recover the bond strength to values statistically similar to control. In contrast, Justin et al[28] showed inability of this procedure in recover the bond strength to suitable mean values. One explanation for this may be related to extended air-drying time (20 s), which can cause dehydration and collapse the collagen scaffold, and different methodologies to test bond strength between studies. The reapplied adhesive layer also showed great efficacy to restore the bond strength; previous report showed this approach as the most reliable method to overcome contamination[19]. When one-step self-etching adhesives were tested on saliva-contaminated dentin, the rinsing with water and air drying approach followed by reapplication of the adhesive restored the μTBS[29].

The similar results found for groups that present any additional strategy may be attributed to features of chosen adhesive system. Darabi et al[19]. suggested that this etch-and-rinse adhesive system contains water as a co-solvent, which gives a lower volatility to this material. Furthermore, it can be suggested that solvents that have a higher affinity to form H-bonds will be able to break the stabilizing H-bonds and other forces that keep the collagen in a shrunken state[12]. Water and ethanol consist of a hydroxyl group that can form strong hydrogen bonds and are both present in composition of used adhesive system[30].

The present study focused on the impact of saliva contamination during restorative procedures on bond strength measuring the dentin bond strength after 1 day of water- aging. Further study should determine the bond strength after long-term water-aging to investigate the effects of saliva contamination on the durability of the dentin-resin bond.

CONCLUSION

Within the limitations, the results of the present investigation suggest that the total- etch two-step adhesive tolerated salivary contamination except when the contamination occurred after application of the bond and it was only removed with an air jet and adhesive system was not reapplied.

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How to cite this article /Como citar este artigo:

Sousa LC, Rodrigues NS, Aquino EYP, Melo MAS, Cunha DA, Saboia VPA. Saliva contamination effects at different application steps on bond strength of total etch two-step adhesive. J Health Biol Sci. 2019 Abr-Jun; 7(2):133-138.